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Glycogen synthase kinase 3: an emerging therapeutic target

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Glycogen synthase kinase 3 (GSK-3) is a serine/threonine protein kinase that has recently emerged as a key target in drug discovery. It has been implicated in multiple cellular processes and linked with the pathogenesis of several diseases. GSK-3 inhibitors might prove useful as therapeutic compounds in the treatment of conditions associated with elevated levels of enzyme activity, such as type 2 diabetes and Alzheimer's disease. The pro-apoptotic feature of GSK-3 activity suggests a potential role for its inhibitors in protection against neuronal cell death, and in the treatment of traumatic head injury and stroke. Finally, selective inhibitors of GSK-3 could mimic the action of mood stabilizers such as lithium and valproic acid and be used in the treatment of bipolar mood disorders.

Protein phosphorylation plays a crucial part in the biochemical control of development and physiology. The regulation of glycogen synthase, the rate-

limiting enzyme in glycogen synthesis, was one of the first examples of complexed multisite hierarchical phosphorylation and has been extensively studied for many years. One of the intriguing outcomes of these studies was the identification of glycogen synthase kinase 3 (GSK-3), a serine/threonine protein kinase that phosphorylates glycogen synthase. More than a decade passed before GSK-3 was recognized as a multifunctional enzyme involved in a broad range of biological processes, including early embryo development in fruit fly and frog, as well as oncogenesis, neurodegenerative disease and cell death. The development of specific inhibitors for

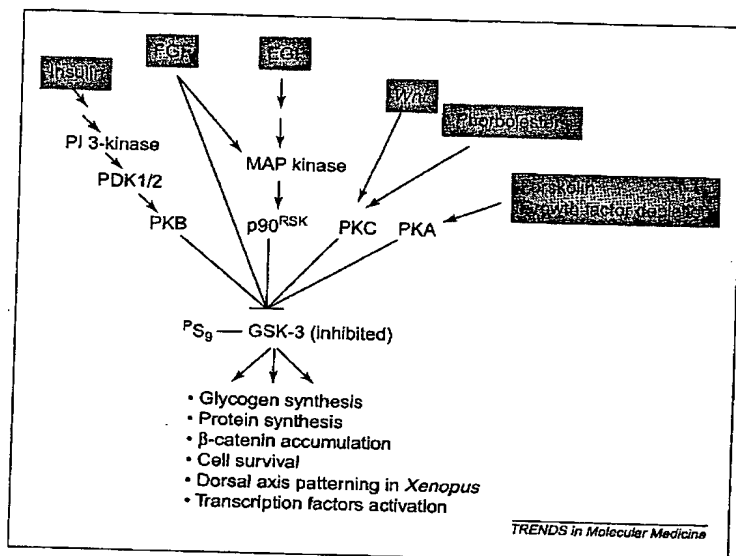


Fig. 1. Regulation of glycogen synthase kinase 3 (GSK-3) by multiple pathways. The diagram shows signaling pathways activated by external stimuli (green). Four protein kinases phosphorylate GSK-3 on Ser9, the key inhibitory site, in intact cells: protein kinase B (PKB), p90^{RSK}, PKC and PKA. Activation of a certain kinase is dependent on the external stimuli. Although signaling pathways converge at GSK-3, inhibition of the enzyme selectively affects specific downstream targets; that is, inhibition of GSK-3 by a certain signal will not lead to activation of all targets, but to a selected target determined by the type of activation. Inhibition of GSK-3 results in several biological responses, as indicated in the figure.

GSK-3 could have therapeutic implications for states of abnormally elevated levels of GSK-3 activity. This review describes the unique properties of GSK-3 and discusses its potential as a therapeutic target.

GSK-3 has unique properties

GSK-3 regulation

The GSK-3 enzyme and its unique properties have been described in recent comprehensive reviews [1–3]. Mammalian GSK-3 exists as two isoforms, α and β , sharing 98% homology in their catalytic domain. Both isoforms are ubiquitously expressed in cells and tissues, and have similar (although not identical) biochemical properties. Unlike most protein kinases, GSK-3 is constitutively active in cells, and its activity can be inhibited by a variety of extracellular stimuli, including insulin, epidermal growth factor (EGF), fibroblast growth factor (FGF) and *Wnt* ligands [1–3]. GSK-3 is inhibited by phosphorylation on a serine residue (Ser9 in GSK-3 β and Ser21 in GSK-3 α) located in the N-terminal domain. Several protein kinases are known to phosphorylate GSK-3 *in vitro*. Subsequently, *in vivo* studies have shown that the enzyme is inhibited by multiple signaling pathways via different protein kinases.

Fig. 1 shows how various extracellular stimuli, including insulin, EGF and phorbol esters, employ different pathways to inhibit GSK-3. These pathways converge at GSK-3, and the question arises as to how specificity for a distinct biological response can be

achieved. The answer probably lies in the fact that additional components, which might work in concert with inhibitory effects of GSK-3, are activated by each of these specific stimuli, thereby leading to specific physiological consequences. For example, inhibition of GSK-3 is important for the activation of glycogen synthase. In certain cell types, such as adipocytes, insulin activates glycogen synthase, whereas EGF does not [4]. Thus, in contrast to EGF, insulin promotes glucose uptake and activates the glycogen-bound type 1 protein phosphatase PP-1G, both of which are required for activation of glycogen synthase [5,6] (Fig. 2a).

Another way in which cross-talk between pathways is limited is by protein complex formation with GSK-3. For example, GSK-3 binds to *Wnt* signaling components such as axin, adenomatous polyposis coli (APC), dishevelled (Dsh) and the GSK-3 inhibitor binding protein GBP/Frat, as reviewed in Refs [1,7]. Complex formation of GSK-3 with some of these components is regulated by *Wnt*, which in turn affects the ability of GSK-3 to enhance β -catenin phosphorylation and degradation [1,7] (Fig. 2b). Association of GSK-3 with different complexes suggests that different GSK-3 'pools' are accessible to distinct cellular pathways, thus allowing specificity of signaling events.

In addition to serine phosphorylation, GSK-3 is phosphorylated on Tyr216, an analogous site to the conserved TEY motif found in the catalytic domain of mitogen-activated protein (MAP) kinase [2,3]. Tyr216 is conserved among all GSK-3 family members including slime mold, yeast and arabidopsis [8]. *In vitro* studies have indicated that the enzyme is autophosphorylated on Tyr216, which increases enzyme activity and classifies it as a dual-specificity enzyme [2,3]. The use of an inactive mutant of GSK-3 has indicated that the enzyme is autophosphorylated on tyrosine residues in intact cells [9]. However, recent work has suggested that, under certain conditions, such as heat shock, transient increase in intracellular calcium, nerve growth factor (NGF) withdrawal, and treatment with staurosporine, GSK-3 tyrosine phosphorylation is increased [10–13]. The calcium-dependent tyrosine kinase PYK2 and a novel cAMP-activated protein tyrosine kinase ZAK1 were shown to phosphorylate GSK-3 β and increase its activity [14,15]. However, their role in the regulation of GSK-3 in intact cells remains to be elucidated.

GSK-3 substrates and function

The fact that GSK-3 is involved in diverse cellular processes suggests that it might have multiple substrates. This is indeed the case. Prior knowledge of the recognition motif of a protein kinase helps the identification of new substrates. The recognition motif of GSK-3 is unconventional: GSK-3 requires pre-phosphorylation of its substrate, meaning that the substrate is first phosphorylated by a 'priming kinase' on a 'priming site' (serine or threonine) that facilitates the subsequent phosphorylation by GSK-3. S¹xxxS²(p)

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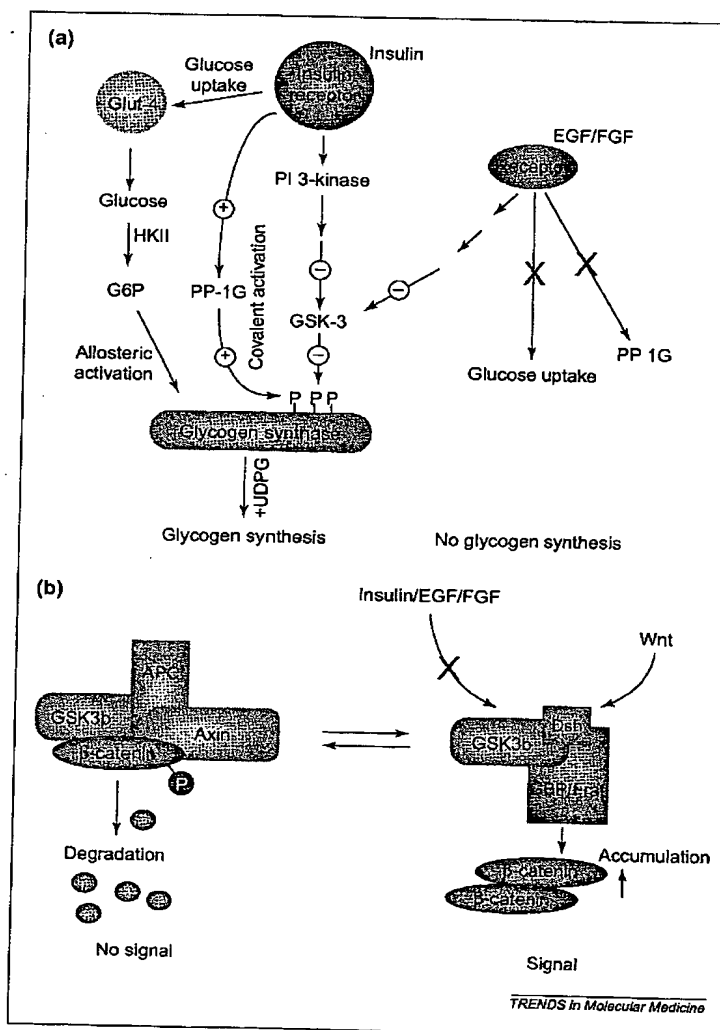


Fig. 2. Specificity of the signaling outcome. (a) In addition to its inhibitory effect on glycogen synthase kinase 3 (GSK-3), insulin promotes glucose entry into cells, where it is metabolized into glucose 6-phosphate (G6P), which acts as an allosteric activator of glycogen synthase (allosteric activation). Insulin also activates the glycogen-bound serine/threonine protein phosphatase PP-1G, which dephosphorylates and activates the enzyme (covalent activation). These events, together with the inhibition of GSK-3, work in concert to activate glycogen synthase and promote glycogen synthesis. Epidermal growth factor (EGF) and fibroblast growth factor (FGF) inhibit GSK-3, but do not promote glucose uptake and activation of PP-1G. Thus, EGF and FGF do not lead to activation of glycogen synthase. (b) When GSK-3 is bound to axin and adenomatous polyposis coli (APC), β -catenin is phosphorylated and degraded. Wnt activates dishevelled (Dsh), which recruits the GSK-3 inhibitor binding protein GBP/Frat and GSK-3. GBP/Frat inhibits GSK-3, and prevents the phosphorylation and degradation of β -catenin. Insulin, EGF and FGF inhibit GSK-3 but do not promote its complexation with Dsh and GBP/Frat. Thus, these factors do not lead to accumulation of β -catenin.

is the 'standard' motif recognized by GSK-3, whereby S^1 is the site phosphorylated by GSK-3, $S^2(p)$ is the 'priming site', and x is any amino acid [8]. However, certain substrates do not follow the $SxxS(p)$ motif or do not require pre-phosphorylation, at least not under *in vitro* conditions: these include c-Jun, c-Myc and c-Myb [8]. One putative explanation for this

inconsistency is that conformational changes in these 'nonpriming' substrates mimic the structure imposed by phosphorylation, and facilitate the interaction of the substrate with the catalytic pocket of the enzyme. Table 1 presents the substrates that were reported to be phosphorylated by GSK-3 and specifies their priming kinase (where possible).

Recent structural studies of GSK-3 crystals have provided further explanation for the preference of phosphorylated substrates by GSK-3 [16,17]. The catalytic loop of the enzyme comprises three positively charged residues that together form a 'positive binding pocket' [16,17], which facilitates efficient binding of a phosphorylated substrate. The preference of phosphorylated substrate also suggests a mechanism for autoinhibition of GSK-3, whereby the phosphorylated N-terminus of the enzyme serves as a pseudosubstrate [9,18].

Whether or not the substrate is pre-phosphorylated, its phosphorylation by GSK-3 inhibits its function. Examples of this include: phosphorylation of glycogen synthase inhibits its activity [8]; phosphorylation of transcription factors c-Jun, CAAT/enhancer binding protein α (C/EBP α) and NF-AT κ inhibits their DNA-binding activity, as reviewed in Ref. [1]; and phosphorylation of insulin receptor substrate 1 (IRS-1) inhibits insulin signaling [19]. These observations support a role for GSK-3 as a key negative regulator of multiple intracellular signaling pathways.

Role of GSK-3 in insulin resistance and diabetes

Insulin resistance is an early change associated with the onset of non-insulin-dependent diabetes mellitus (NIDDM) or type 2 diabetes. The major defect in insulin resistance is the inability of peripheral tissues, such as muscle, liver and fat, to respond normally to physiological concentrations of insulin. Since one of the major characteristics of diabetic muscle is the severe inhibition of glycogen synthesis and the loss of glycogen synthesis [20], it is reasonable to assume that a defect in GSK-3 regulation might be associated with insulin resistance. Furthermore, a role for GSK-3 in regulating adipogenesis was proposed in view of its ability to phosphorylate C/EBP α [21]. This transcription factor controls the expression of numerous genes and is required for pre-adipocyte differentiation, mainly through induction of peroxisomal proliferator-activated receptor γ (PPAR γ), a key activator of adipogenesis [22]. It was shown that insulin-induced dephosphorylation of C/EBP α involved inactivation of GSK-3, and that treatment with the GSK-3 inhibitor lithium prevented differentiation of 3T3L1 pre-adipocytes into adipocytes [21]. Thus, GSK-3 might be an important mediator of adipose accumulation in insulin resistance conditions.

The involvement of GSK-3 in diabetes was further demonstrated in two model systems: in fat tissue of obese diabetic mice, where GSK-3 activity was found to be twofold higher than in control mice [23]; and in

Table 1. Substrates of glycogen synthase kinase 3 (GSK-3)

| Protein | Sequence | Priming kinase |
|-------------------|----------------------------------|-------------------------|
| Glycogen synthase | RPASVPPSPSLSRHSSPHQSEDEE | CK-2 |
| CREB | KRREILSRPPSYR | PKA, p90 ^{Rsk} |
| elF2B | EELDSRRGSPQLDDIK | MAP kinase |
| HSF-1 | KEEPPSPQSPR | DYRK1A |
| C/EBP α | TPPPTVPVSP | MAP kinase |
| NF-ATc | Multiple sites at the N-terminus | Not known |
| Inhibitor-2 | GLMKIDEPSTPYHSMIGDDDDAYS | PKA |
| c-Jun | KEEQQTVPMPGETPPLSPIDMESQER | CK-2 |
| c-Myc | DIWKKFELIPTPLSPRSRRSG | Not required |
| c-Myb | DIWKFELVSPPTSPPWGL | Not required |
| β -catenin | SYLDSGIHSGATTAPSLSGKG | Not required |
| tau | Multiple sites | Not required |
| IRS-1 | Multiple sites | Not known |

Residues identified as phosphorylation sites for GSK-3 are blue. Residues identified as priming sites are red. The identity of the priming kinase is indicated.

Abbreviations: CREB, cAMP response binding protein; C/EBP α , CCAAT/enhancer binding protein α ; HSF-1, heat shock factor-1; elF2B, eukaryotic protein synthesis initiation factor 2B; NF-ATc, nuclear factor of activated T-cells; IRS-1, insulin receptor substrate 1; DYRK, dual-specificity tyrosine phosphorylated and regulated kinase.

skeletal muscle of patients with type 2 diabetes, where GSK-3 activity and expression levels were significantly higher than in healthy individuals [24]. Thus, in insulin-sensitive tissue directly involved in the pathogenesis of type 2 diabetes, GSK-3 activity is higher than normal and presumably contributes to impairment of insulin action.

Recently, GSK-3 was shown to phosphorylate IRS-1, which in turn attenuates insulin signaling [19]. IRS-1 is the immediate substrate of insulin receptor tyrosine kinase, which phosphorylates the protein on multiple tyrosine residues in response to insulin [25]. In addition, IRS-1 is predominantly phosphorylated on serine/threonine residues in the absence of stimuli [26]. It appears that this type of phosphorylation of IRS-1 converts the protein from a positive to a negative regulator of insulin receptor signaling (Fig. 2a). Tyrosine phosphorylation of IRS-1 is a positive trigger of insulin action, which initiates numerous signaling components including phosphoinositide 3-kinase (PI 3-kinase), protein kinase B (PKB) and MAP kinase [25,27,28]. Yet, its serine/threonine phosphorylation results in the opposite effect, presumably by direct interaction of IRS-1 with the insulin receptor (Fig. 3a). This was demonstrated by two studies. In the first study, treatment with okadaic acid, a protein phosphatase inhibitor, followed by hyperphosphorylation of IRS-1 on serine/threonine residues, led to inhibition of the insulin signaling pathway [29]. In the second study, the cytokine tumor necrosis factor α (TNF- α), which mediates insulin resistance, was shown to activate serine phosphorylation of IRS-1 and inhibit insulin-induced tyrosine phosphorylation of the insulin receptor [30]. These studies implicated serine/threonine protein kinases as important regulators in insulin resistance.

GSK-3 was the first protein kinase identified as an IRS-1 kinase [19]. Phosphorylation of IRS-1 on

multiple serine residues by GSK-3 impaired insulin receptor tyrosine kinase activity and insulin action in intact cells [19]. This notion fits well with the fact that GSK-3 is constitutively active and phosphorylates IRS-1 in the absence of stimulus. Thus, GSK-3 serves as a 'gatekeeper' to limit activation of insulin receptor signaling. In the absence of insulin, GSK-3 maintains the phosphorylation state of the multiple serine residues on IRS-1, thereby limiting insulin receptor signaling (Fig. 3b). In the presence of insulin, GSK-3 is inhibited, and tyrosine phosphorylation of IRS-1 mediates the downstream insulin signaling pathway (Fig. 3b). Later studies identified additional serine/threonine protein kinases that were capable of phosphorylating IRS-1, including PKC, Jun kinase (JNK) and PKB [31-33]; however, their role in insulin resistance and diabetes remains to be clarified.

GSK-3 in Alzheimer's disease

GSK-3 and tau

GSK-3 interacts with several neuronal proteins that are directly linked with Alzheimer's disease (AD). The microtubule-associated protein tau, a neuron-specific structural protein abundant in the brain, is a major component of neurofibrillary tangles, which are an intraneuronal aggregate of paired helical filaments (PHFs) and one of the most notable neuropathological hallmarks of AD, as reviewed in Ref. [34]. The tau protein in neurofibrillary tangles (also termed PHF-tau) is hyperphosphorylated on serine and threonine residues, and this is believed to be the primary cause of the generation of AD tangles. GSK-3 was shown to phosphorylate tau both *in vitro* and in intact cells on multiple sites, some of which are aberrant in the abnormally hyperphosphorylated tau [35,36]. Moreover, cells co-transfected with tau and GSK-3 β showed abnormal microtubule bundles, which could be dissociated by colchicine, in contrast with microtubules seen in cells transfected with tau alone [37].

Recent animal studies have provided further support for the view that GSK-3 is the most likely candidate for the protein kinase responsible for the abnormal phosphorylation state of tau in AD brain. Immunohistochemical studies located GSK-3 in neurofibrillary tangles [38]. Moreover, GSK-3 β is elevated in AD human brain [39]. In conditional transgenic mice overexpressing GSK-3 in the brain, tau was hyperphosphorylated and neurons exhibited abnormal morphology [40]. Notably, different conclusions were drawn from studies in transgenic mice overexpressing a constitutively active GSK-3 mutant in brain and spinal cord [41]. Despite the observation of hyperphosphorylation of tau, no aggregates of PHF or tangles were formed [41]. This emphasizes that extremely subtle balanced phosphorylation of tau protein governs the neuropathological changes taking place in AD brain tissues. However, these studies do not refute the importance of inhibition of GSK-3 as a therapeutic approach to treat AD.

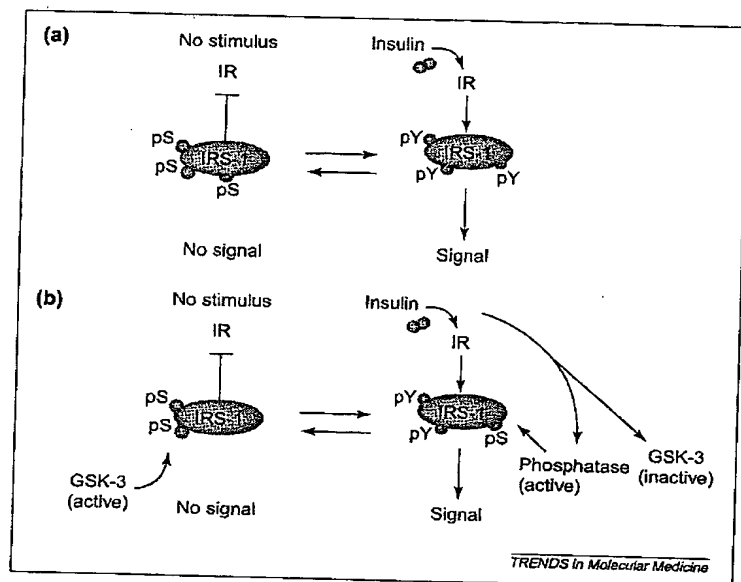


Fig. 3. Insulin receptor substrate 1 (IRS-1) phosphorylation regulates insulin signaling. (a) Phosphorylation of IRS-1 on serine residues (shown as pS) in a non-stimulating situation converts IRS-1 to a negative regulator of the insulin receptor (IR); by contrast, in the presence of insulin, IRS-1 is phosphorylated on tyrosine residues (pY) and mediates the insulin signaling pathway. (b) Glycogen synthase kinase 3 (GSK-3) is constitutively active in the absence of stimulus and phosphorylates IRS-1 on serine residues. GSK-3 serves as a gatekeeper to limit activation of insulin receptor signaling. In the presence of insulin, IRS-1 is tyrosine phosphorylated, GSK-3 is inhibited, and a protein phosphatase (PP-1G) is activated, leading to activation of the insulin signaling pathway.

GSK-3 and the amyloid β peptide

In addition to tau, an important link has been demonstrated between the amyloid β peptide ($A\beta$), which is another AD hallmark, and GSK-3. This small proteolytic product (39–41 amino acids) of the amyloid precursor protein (APP), a ubiquitous transmembrane protein that is expressed by numerous cell types, is accumulated in AD senile plaques and is toxic towards neurons [34]. Recent work has suggested that $A\beta$ peptide induces GSK-3 activation and tau hyper-phosphorylation, providing a potential mechanistic link between neurofibrillary tangle formation and the $A\beta$ peptide accumulation, and unifying the two most notable pathologies of AD [42].

GSK-3 and the presenilins

An intriguing interaction has been described between GSK-3 and the presenilin proteins. Mutations in the genes encoding presenilin-1 and -2 are associated with the majority of autosomal dominant familial cases of AD [34]. Takashima and colleagues first reported that presenilin-1 binds directly to GSK-3 and tau, and that mutated presenilin-1 had an increased capacity to bind GSK-3 [43,44]. However, other studies have suggested that mutated presenilin-1 has a decreased capacity to bind GSK-3 [61]. Although the role of presenilin mutations in AD pathology has not been elucidated, it is now recognized that presenilin-1 can alter GSK-3 activity and/or its ability to phosphorylate tau.

Even more intriguing is the observation that presenilins interact with the β -catenin key signaling components in the *Wnt*-activated pathway [44,45]. Studies in cells overexpressing wild-type presenilin-1 or its mutant form showed that both wild-type and mutant presenilin-1 directly interacted with β -catenin and, moreover, that cytoplasmic levels of β -catenin protein were reduced compared with control cells [44]. Thus, presenilin-1 might act as a scaffold protein that brings GSK-3 into proximity with its substrates tau and β -catenin. However, it is not yet clear whether presenilins play a physiological role in the generation of neurofibrillary tangles through activation of GSK-3, tau phosphorylation and/or modulation of β -catenin levels.

GSK-3 and cell fate

In the past few years, GSK-3 has been shown to facilitate apoptosis, providing further evidence of the link between GSK-3 and AD. The pro-apoptotic feature of GSK-3 might reflect the notion that insulin and its downstream targets, PI 3-kinase and PKB, as well as *Wnt* ligands, are acknowledged survival factors [7,46]. Indeed, the pro-apoptotic role of GSK-3 has been shown in several studies: overexpression of GSK-3 in pheochromocytoma (PC12) cells was sufficient to promote cell death [47]; activation of GSK-3 in cerebellar granule neurons led to premature cell death [48]; and GSK-3 facilitated staurosporine-induced cell apoptosis in human neuroblastoma cells (SH-SY5Y) [49]. Moreover, GSK-3 inhibition protected against cell death, as shown by the following findings: neuronal expression of Frat1, an endogenous GSK-3 β inhibitor, rescued cell death induced by inhibition of PI 3-kinase [50]; treatment of primary neurons with synthetic inhibitors of GSK-3 (SB-216763 or SB-415286, see below) protected against cell death induced by a reduction in the activity of the survival factor PI 3-kinase [51]; and inhibition of GSK-3 by lithium also protected neurons from apoptosis [49]. Together, these studies indicate that GSK-3 is a key player in determining neuronal cell fate. However, it appears that inhibition of GSK-3 might not be sufficient for protecting against cell death induced by certain other pro-apoptotic pathways such as NGF withdrawal [50].

Different observations on the role of GSK-3 in cell fate were obtained from studies of GSK-3 β -knockout, which suggested a requirement for GSK-3 in cell survival [52]. These studies showed that disruption of the gene encoding GSK-3 caused embryonic lethality owing to massive apoptosis of hepatocytes [52]. However, these results might not be contradictory after all. Clearly, GSK-3 activity is crucial for the well-being of the cell and its complete absence leads to death. On the other hand, an uncontrolled hyperactive enzyme might also be lethal, and neurons might be more sensitive than other cell types to GSK-3 overactivity. It is pertinent in this regard that GSK-3 can be activated by two independent mechanisms, namely: dephosphorylation of a serine

residue or phosphorylation of a tyrosine residue. Activation of the enzyme through tyrosine phosphorylation or decreased serine phosphorylation might lead to distinct signaling consequences. Another outstanding question in this respect concerns the existence of possible cross-talk between these two phosphorylated sites, and whether it leads to different cellular responses such as apoptosis versus survival.

GSK-3 and bipolar disorder

A notable recent development is the implication of a role for GSK-3 in psychiatric disorders. Lithium ions, used as a primary mood stabilizer in the chronic treatment of patients with bipolar disorder, were shown to be a selective inhibitor of GSK-3, as reviewed in Ref. [53]. A series of studies demonstrated that lithium ions mimic the loss of GSK-3 activity in intact cells. Lithium was shown to cause activation of glycogen synthesis [54], stabilization and accumulation of β -catenin [55], induction of axis duplication in *Xenopus* embryo [53], and protection against neuronal cell death [49,56]. Notably, a study on GSK-3 expression in post mortem frontal cortex tissues from bipolar disorder patients failed to document altered enzyme levels [57]. However, such observations do not negate a role for GSK-3 dysregulation in the pathophysiology of bipolar disorder and related mood disorders, since total GSK-3 expression levels do not reflect its true *in situ* levels of activity.

It is also noteworthy that lithium ions are not selective for GSK-3 inhibition: lithium also inhibits inositol monophosphatase (IMPase) and could interfere with the inositide cycle [53]. Further support for a role of GSK-3 in bipolar disorder is the finding that valproic acid, another drug commonly used in this disorder, inhibits GSK-3 activity in relevant therapeutic concentrations *in vitro* and in human neuroblastoma cells [56]. Taken together, there is compelling evidence that the primary molecular target for the mood-stabilizing agents lithium ions and valproic acid is GSK-3 and, hence, it is likely that some defects in the neuronal regulation of GSK-3 activity are implicated in bipolar disorder.

GSK-3 inhibitors: a potential therapeutic approach

The paradigm that GSK-3 acts as a suppressor in signaling pathways points to a potential role for GSK-3 inhibitors in therapeutic interventions. Such inhibitors might be useful in pathological conditions associated with abnormally increased levels of GSK-3

activity such as type 2 diabetes and AD. In addition, specific inhibitors of GSK-3 mimic the therapeutic action of the mood stabilizers lithium and valproic acid and might therefore be plausible drugs in treating patients with bipolar disorder. Furthermore, the pro-apoptotic property of the enzyme suggests a potential use of such inhibitors in the treatment of head trauma and stroke.

Several synthetic GSK-3 inhibitors have been reported. Two structurally distinct compounds, SB-216763 and SB-415286, developed by Glaxo Smith Kline, were shown to inhibit the enzyme with an IC_{50} (i.e. the concentration of inhibitor required for 50% inhibition of activity) in the nanomolar range [51]. Treatment with these inhibitors activated glycogen synthesis and protected against neuron death [51,58]. Other small-molecule inhibitors were recently patented by Chiron (Emeryville, CA, USA) and proposed to be plausible drugs for the treatment of type 2 diabetes. *In vitro* exposure of GSK-3 inhibitors to isolated skeletal muscles of Zucker diabetic rats enhanced insulin-induced stimulated glucose transport [59]. Also of interest is the active ingredient of the traditional Chinese medicine for chronic myelocytic leukemia, indirubin, which was shown to inhibit GSK-3 and to arrest cell growth. However, indirubin is not a selective GSK-3 inhibitor in that it also inhibits cyclin-dependent protein kinase 2 (CDK-2) [60].

The above GSK-3 inhibitors are ATP competitive, and their specificity was determined *in vitro* within the range of kinases tested. Thus, their *in vivo* specificity remains to be validated. As inhibition of GSK-3 leads to accumulation of β -catenin, which has been linked with the occurrence of colon cancer and melanoma [7], there is a concern that these inhibitors carry a risk of promoting cancer. On the other hand, chronic treatment with lithium has not been reported to cause cancer. Controlled administration of appropriate doses of GSK-3 inhibitors, possibly in combination with tissue-targeting design, might be a safer approach in the use of these inhibitors. In the future, it might be desirable to develop inhibitors that will be capable of affecting distinct cellular targets, for example glycogen synthase (a primed substrate) without affecting β -catenin (a nonprimed substrate).

In summary, the emerging field of GSK-3 inhibitors continues to grow, and it is becoming evident that GSK-3 is a promising therapeutic target. Its unique biochemical properties distinguishing it from most protein kinases should enable development of specific and selective drugs.

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